High-throughput FISH *on chips*

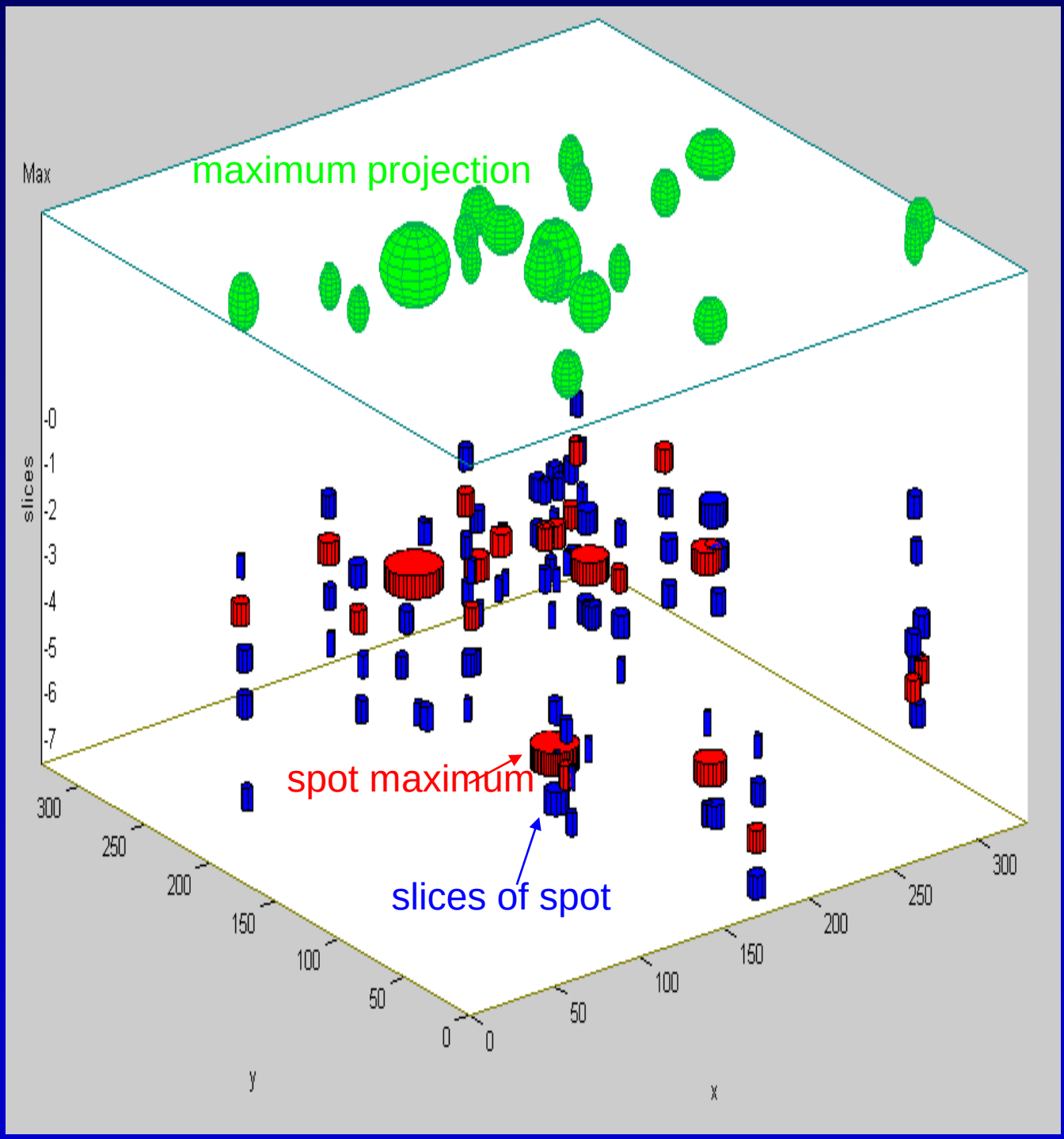
Towards automated digital image analysis of genetic alterations in thousands of tumors with multiple probes

How accurate is the analysis of low level gene copy number gains or deletions by FISH on tissue sections?

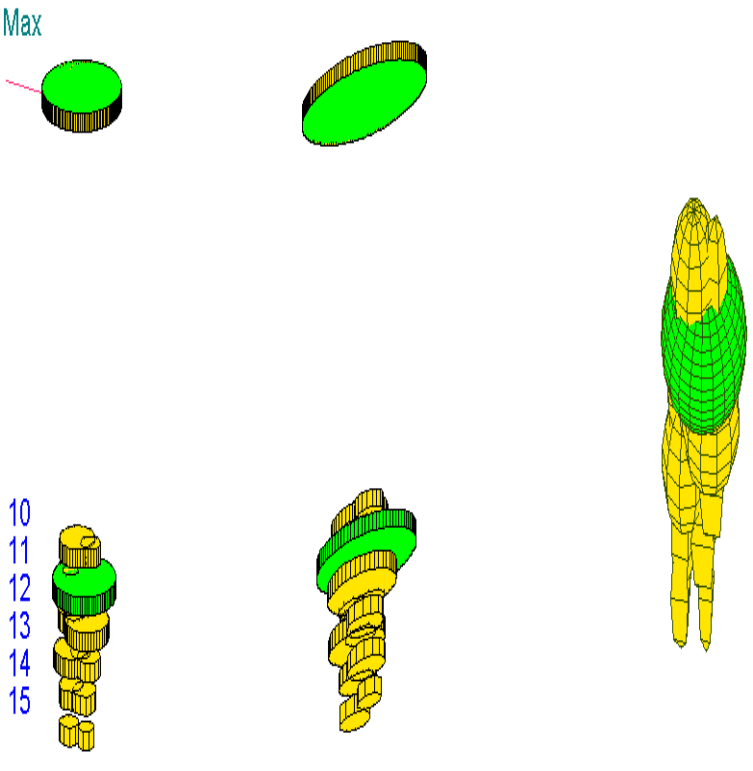
Problems of automated FISH spot counting

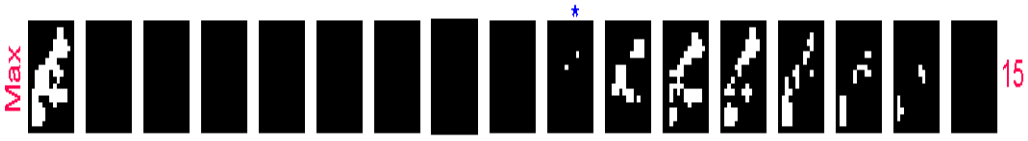
- Signals at different focal planes (3-D)
- Definition of nuclear boundaries
- No reliable algorithms available

3D view of 22 FISH spots in tissue sec



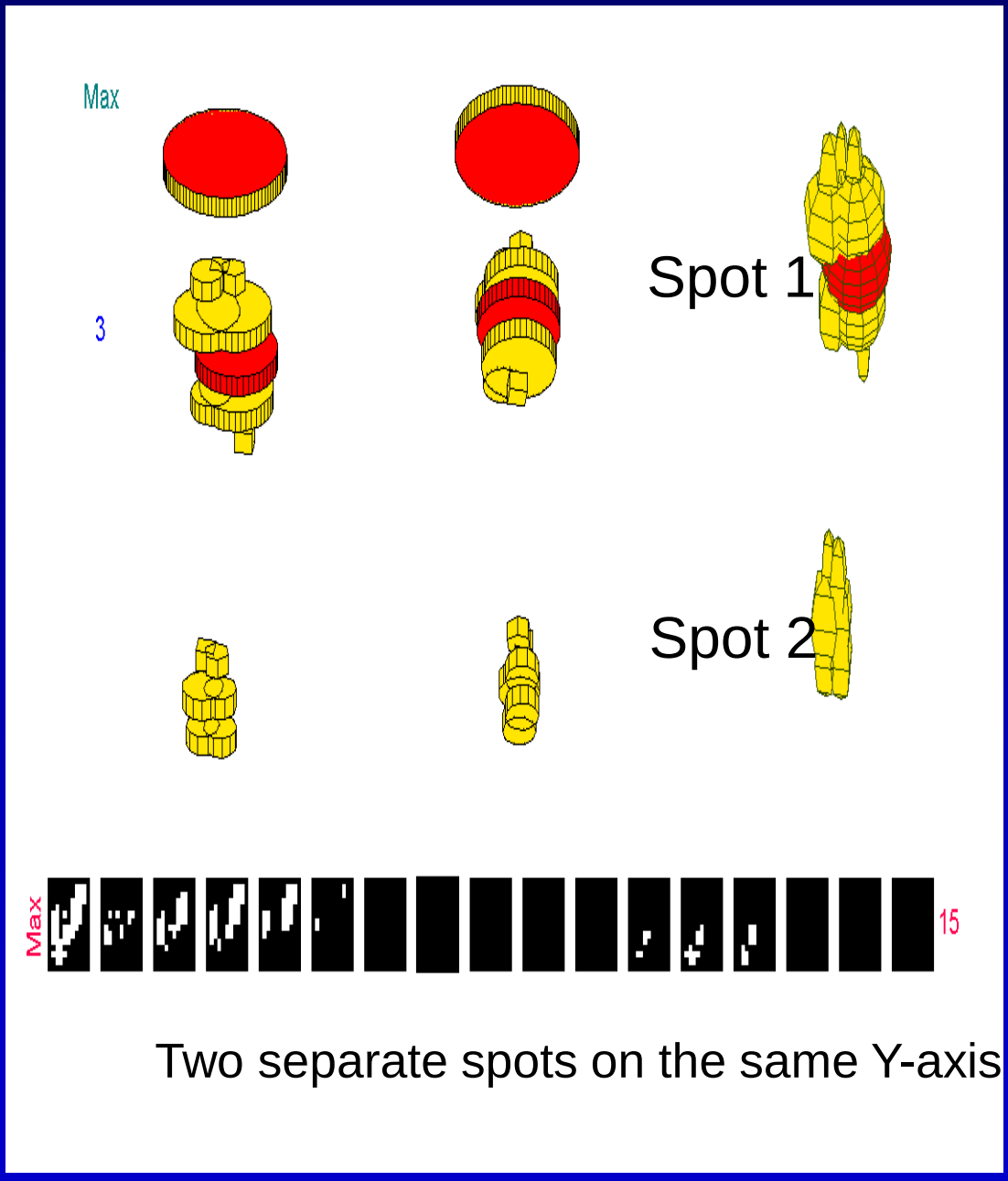
3D reconstruction of FISH spot





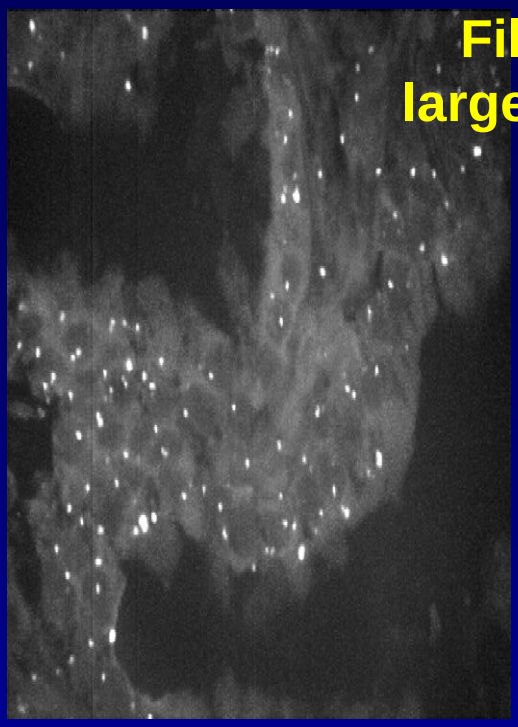
one single spot appearing on 6 confocal slices

3D reconstruction of FISH spot

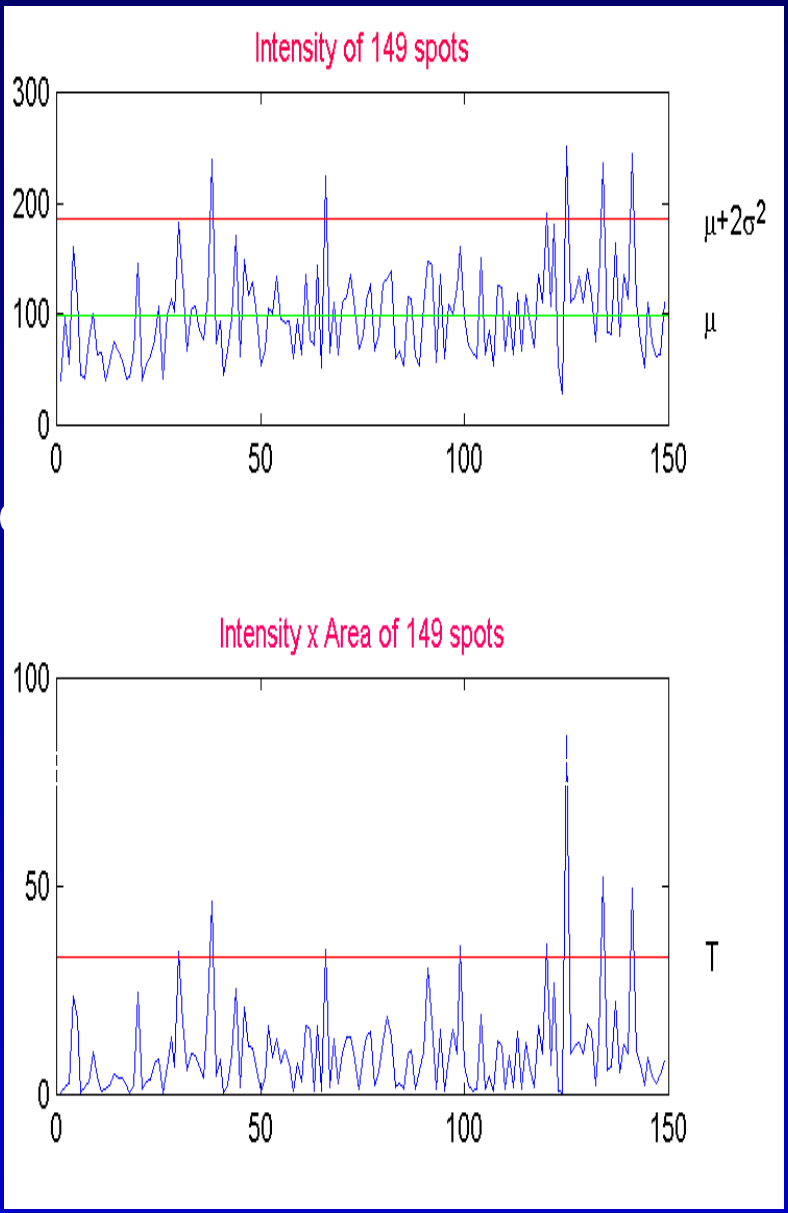


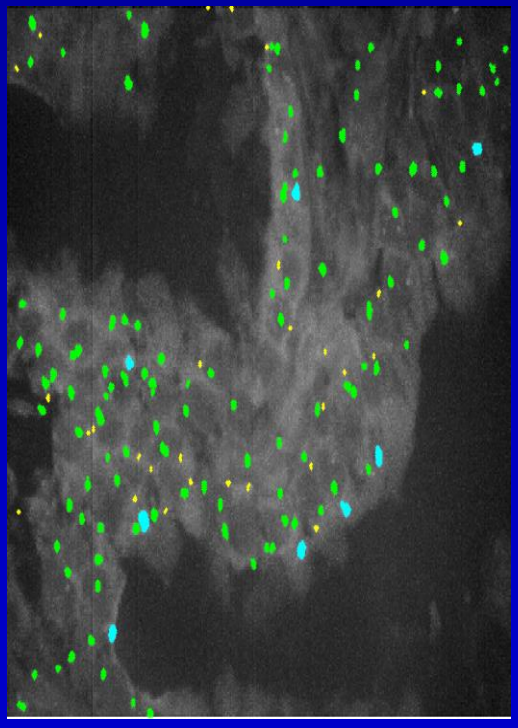
Two separate spots on the same Y-axis

Filtering for elimination of small and large particles by spot area and intens



Xcen probe, normal pr





- true FISH signals
- small autofluorescent tissue p
- large autofluorescent tissue p

Acquisition of confocal images

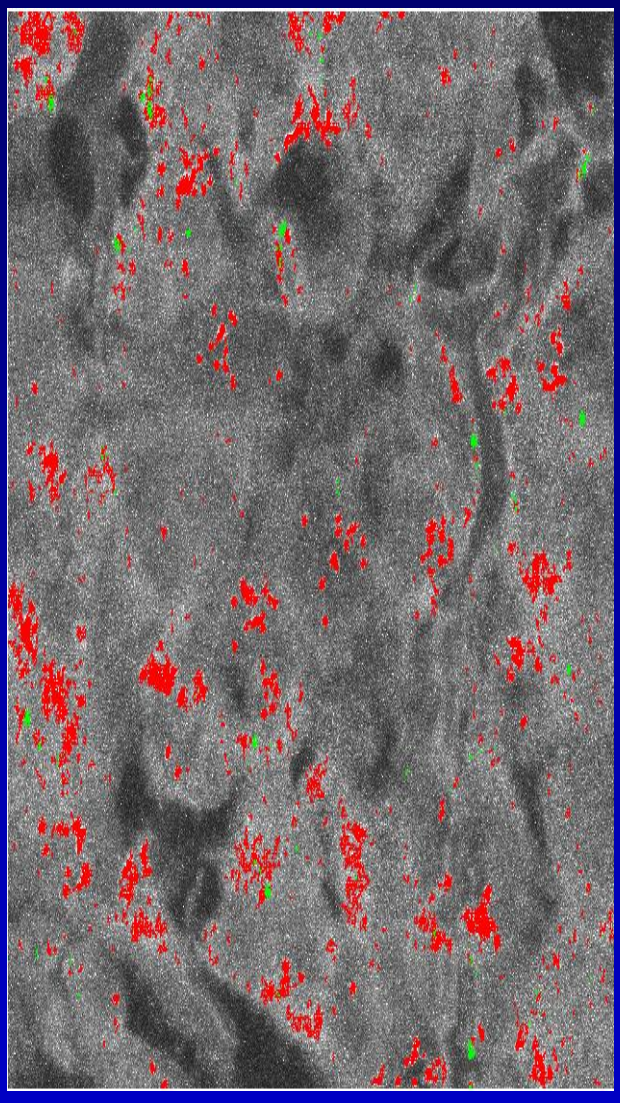
CARV non-laser confocal module

Laser scanning microscopes

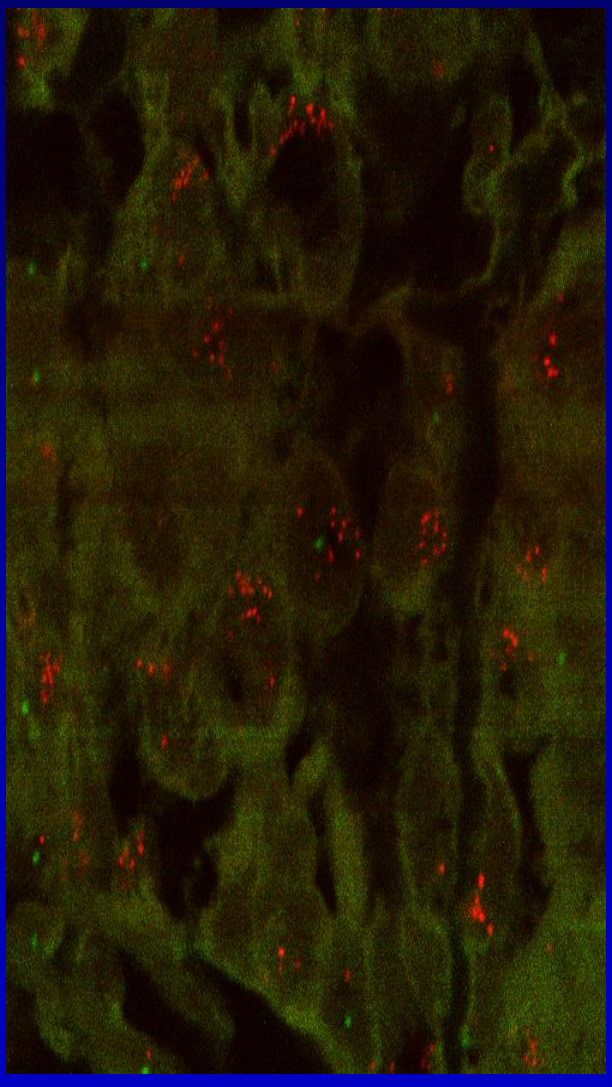
ww.atto.com

ww.zeiss.com

Automated spot recognition



spot recognition
(max. image)

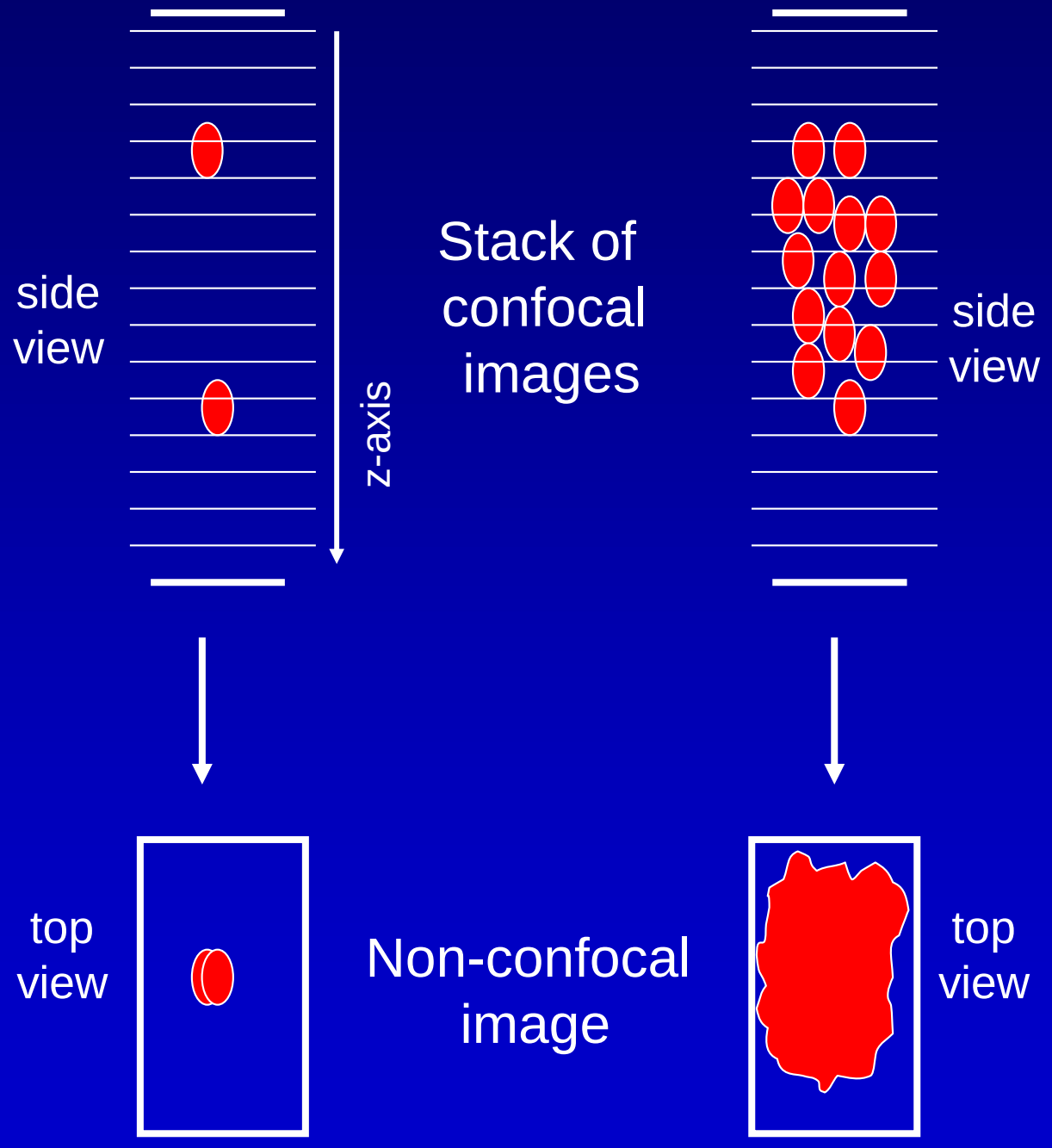


confocal image

Accuracy of automated spot counting in tissue sections

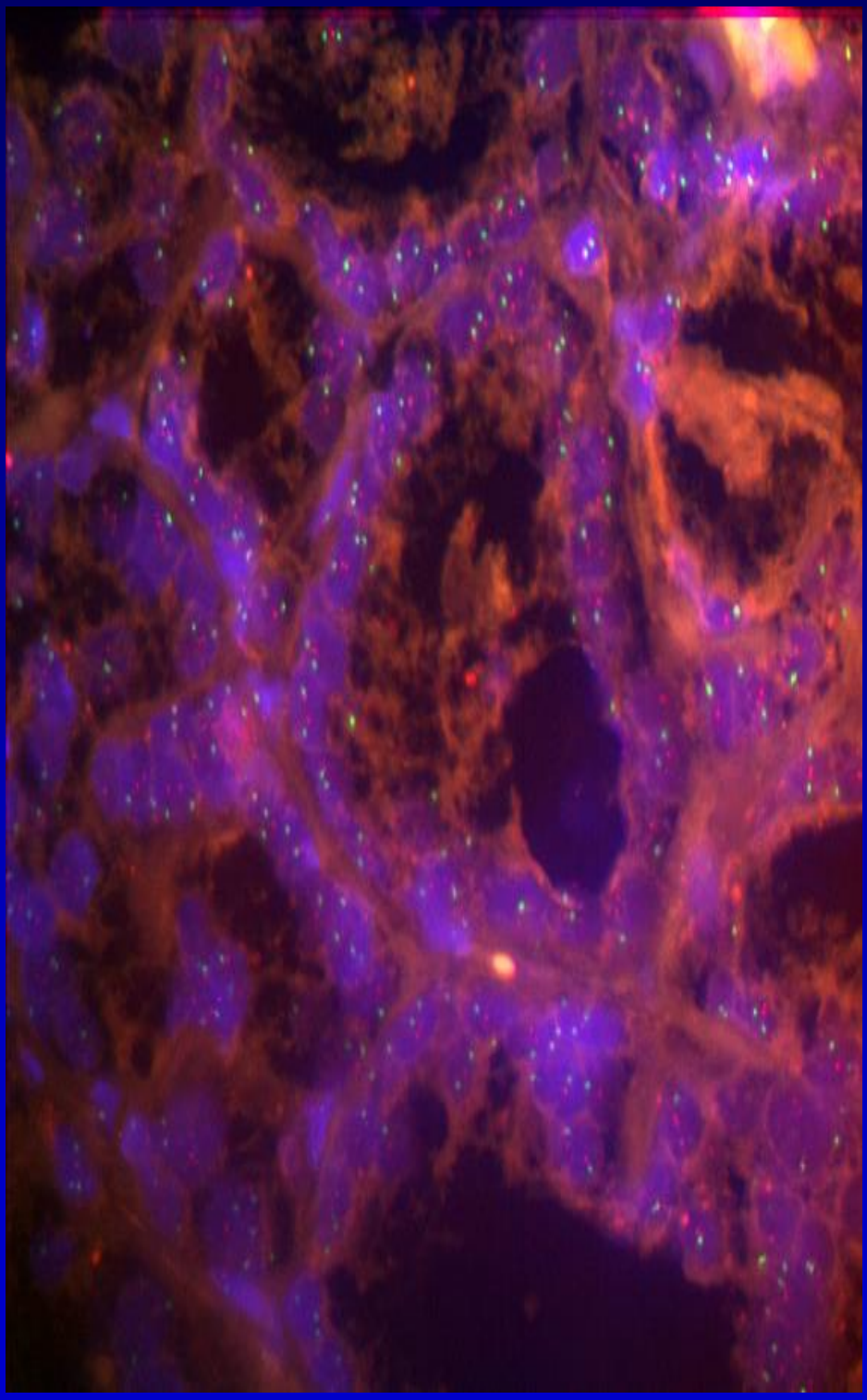
specimen	FISH probes	Red/gr. spots	autom. ratio#	manual ratio*
Norm. prostate	AR/Xcen	95/96	1.0	1.0
Norm. prostate	AR/Xcen	124/126	1.0	1.0
Norm. prostate	AR/Xcen	92/91	1.0	1.0
Prostate cancer	HER2/17cen	212/199	1.1	1.0
Breast cancer	17q23/17cen	763/79	9.7	10.6

Z-axis scanning: Identification of all individual spots in a tissue section



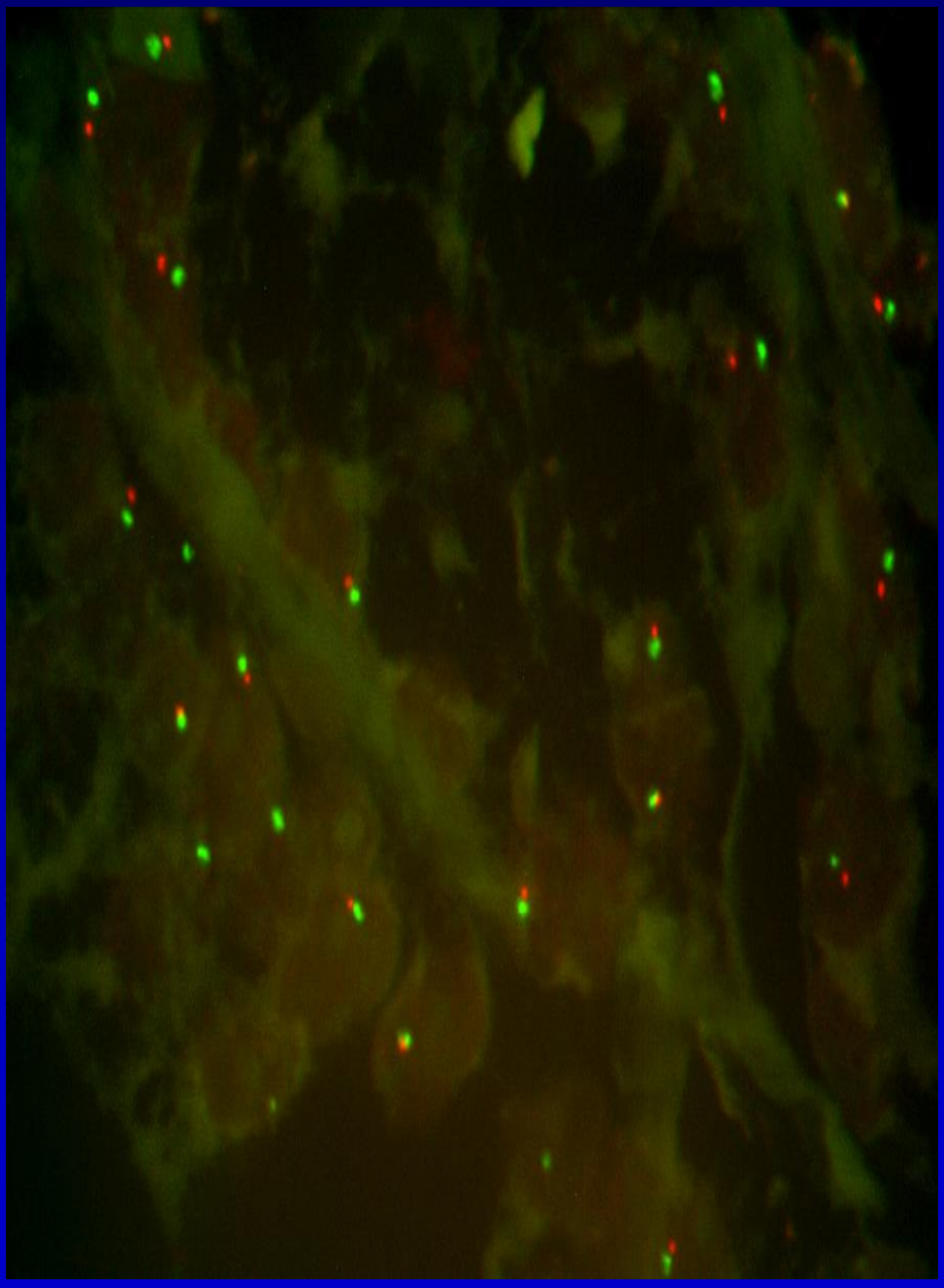
Her-2/17cen FISH in prostate cancer

max. projection from image stack

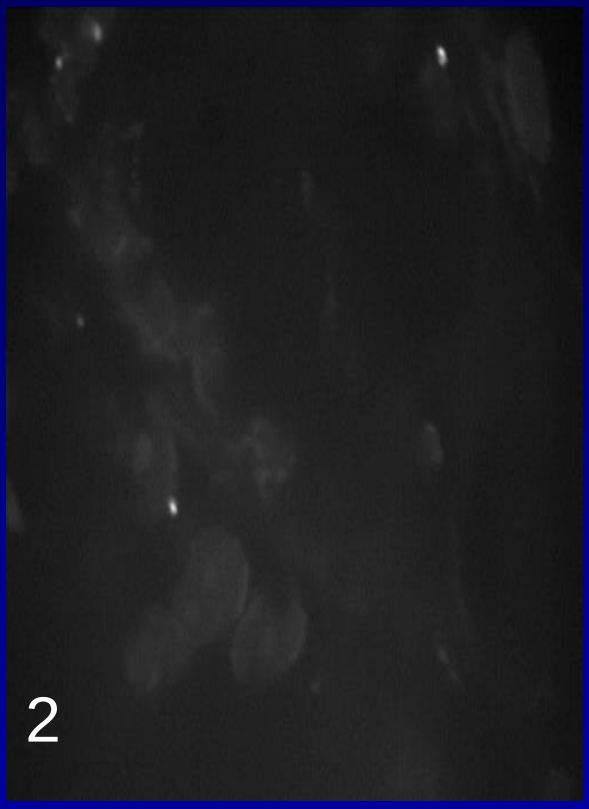


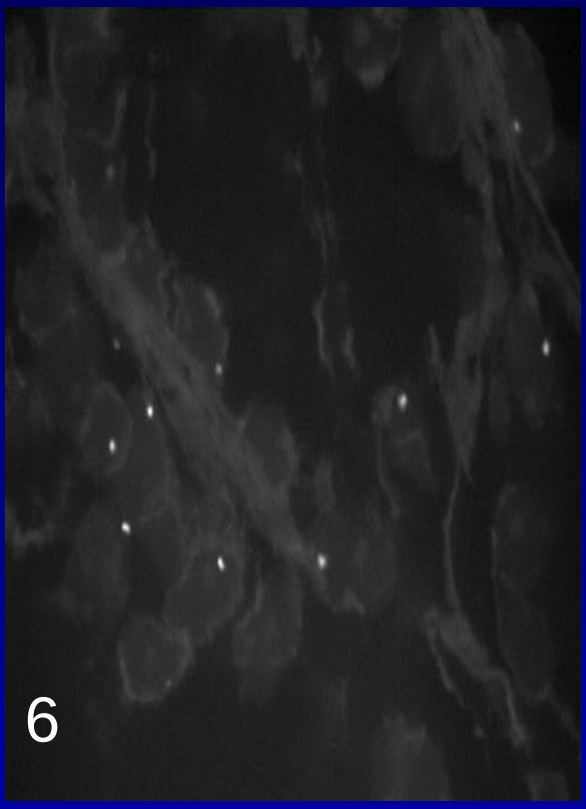
AR/Xcen FISH in normal prostate

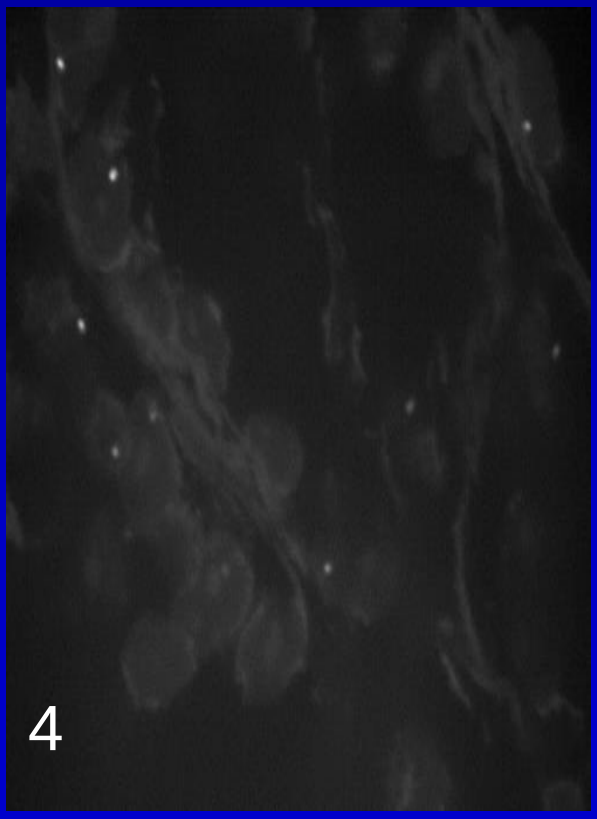
max. projection from image stack

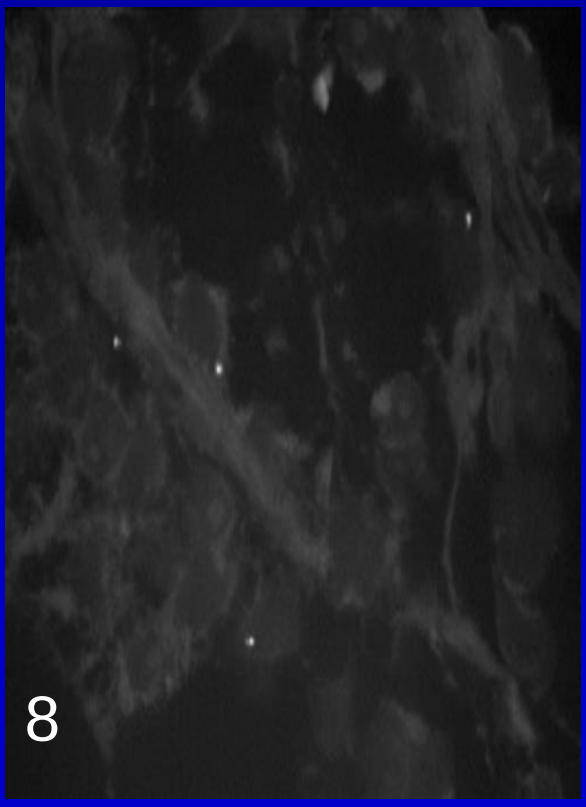


Stack of confocal FISH images (Xcen)

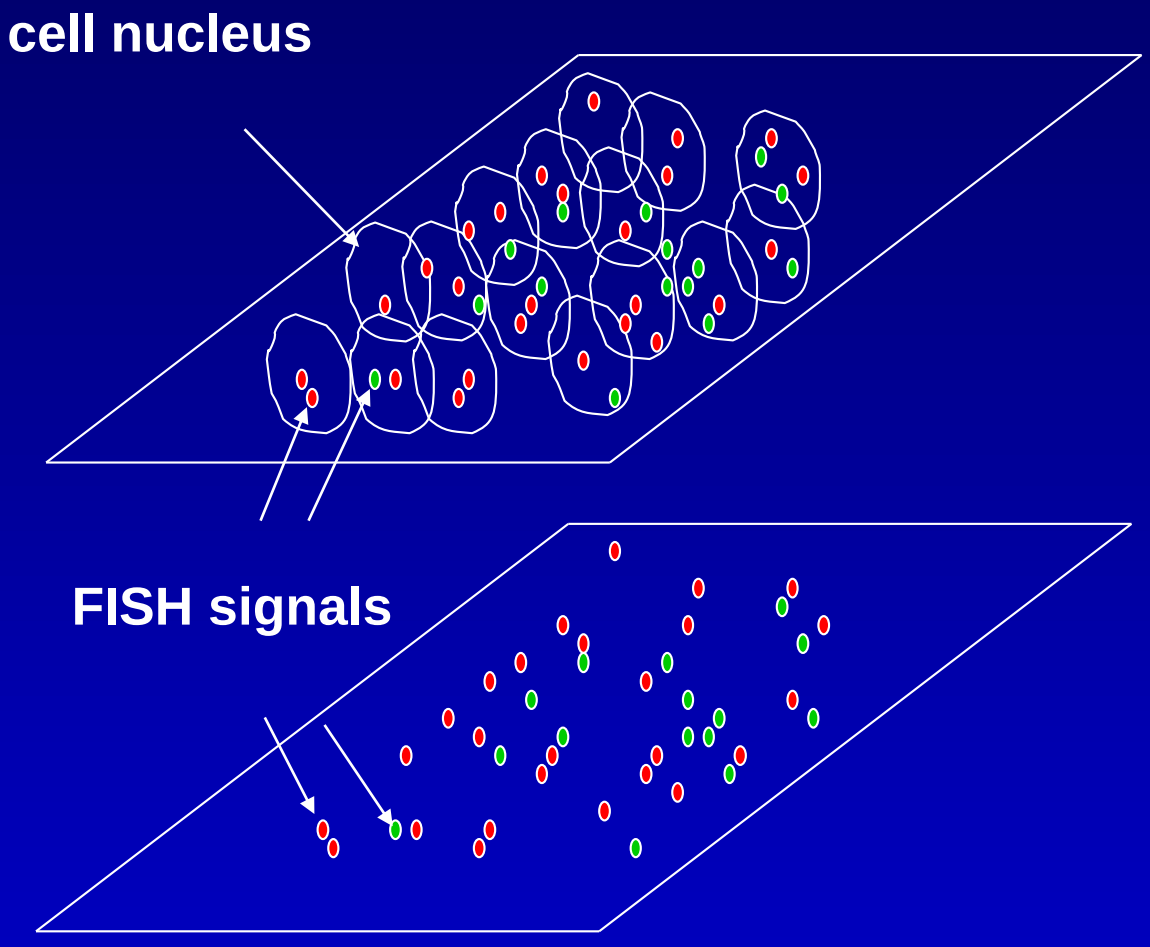






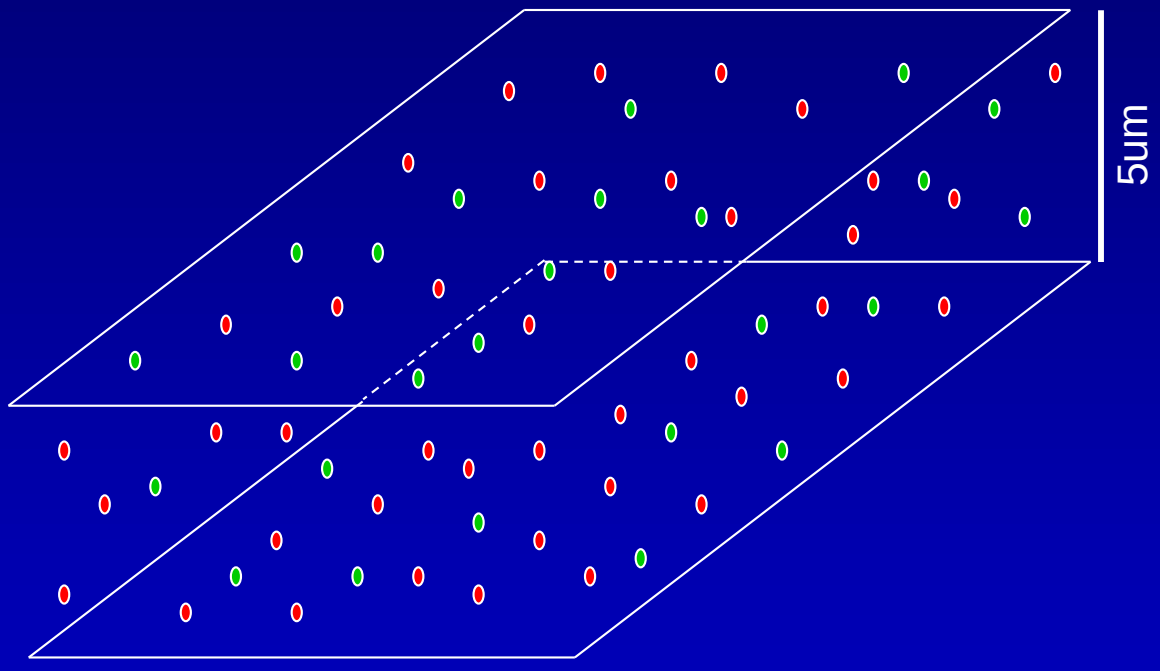


Automated FISH-spot counting on tissue sections



1. Traditional signals-per-nucleus approach ---> overall test to-reference probe ratio in a defined tumor area.

Automated FISH-spot counting on tissue sections



2. spot identification and counting from consecutive series of confocal microscope images.

Summary

- New strategy for FISH spot counting based on counting the ratio of test and reference spots in a field of view
- Confocal imaging and 3D spot counting --> automated and more accurate FISH analysis

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